REMARKS

Claims 1-25 were pending at the time of this Non-Final Office Action dated August 11, 2009. Claims 10-25 were alleged as lacking unity with elected matters and withdrawn from consideration. Claims 1-9 have been considered by the Office, and were rejected.

Claims 1 and 5 have been amended and claims 6-9 have been canceled without prejudice or disclaimer. The amendments add no new matter to the application. Support for the amended claim 1 can be found, *inter alia*, in the original claims 1, 6 and 9. Support for the amended claim 5 can be found, *inter alia*, in the original claim 5.

With respect to the amendments, Applicants have not dedicated or abandoned any unclaimed subject matter and have not acquiesced to any rejections and/or objections made by the Patent Office. Applicants expressly reserve the right to pursue prosecution of any presently excluded subject matter or claim embodiments in one or more future continuation and/or divisional application(s).

Upon entry of the amendments, claims 1-5 will be pending and under examination. Entry of the amendments and reconsideration in view of the following comments are respectfully requested.

Priority

The Examiner stated that the present application is not entitled to the priority date of March 26, 2004 based on the Chinese Application No. 200410029590.7 because Applicants allegedly have not filed a certified copy of the Chinese Application as required by 35 U.S.C. § 119(b). (OA at page 2.)

Applicants respectfully submit that a certified copy of the Chinese Application No. 200410029590.7, filed on March 26, 2004, was submitted to the Office on September 22, 2006. Included in this response is a certified English translation of the foreign priority document as

Exhibit A. Therefore, the present application should be entitled to the priority date of March 26, 2004.

Claim Objections

Claims 5 and 6 were objected to because of informalities. (OA at page 3.) Claim 5 has been amended to correct the informalities. Claim 6 has been canceled without prejudice or disclaimer. Therefore, this objection should be properly withdrawn.

Claim Rejections - 35 U.S.C. § 112

Claims 7 and 8 were rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. (*Id.*) Claims 7 and 8 have been canceled without prejudice or disclaimer. Therefore, this rejection becomes moot.

Claim Rejections - 35 U.S.C. § 102

Du et al.

Claims 1-3, 5-7 and 9 were rejected under 35 U.S.C. § 102(a) as allegedly being anticipated by Du et al. alone or as evidenced by O'Neil et al. regarding the molecular weight of the compounds utilized by Du et al. (OA at page 4.) The Examiner asserted that Applicants cannot rely upon the foreign priority papers to overcome this rejection because a certified copy of the Chinese Application No. 200410029590.7 has not been received and a translation of said papers has not been made of record in accordance with 37 C.F.R. § 1.55. (*Id.*)

As discussed above, the present application claims priority to the Chinese Application No. 200410029590.7, filed on March 26, 2004. Therefore, the present application should be entitled to the priority date of March 26, 2004, which antedates the publication date of November

24, 2004 of Du et al. Accordingly, Du et al. does not qualify as prior art under 35 U.S.C. § 102(a) and this rejection should properly be withdrawn.

Knecht et al.

Claims 1-7 and 9 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Knecht et al., alone or as evidenced by O'Neil et al. regarding the molecular weights of the compounds utilized by Knecht et al. (OA at page 6.) Applicants respectfully traverse this rejection.

The legal standard for anticipation under 35 U.S.C. § 102 is one of strict identity. *Trintec Industries, Inc. v. Top-U.S.A. Corp.*, 63 U.S.P.Q.2d 1597 (Fed. Cir. 2002). To anticipate a claim, a single prior source must contain each and every limitation of the claimed invention. *In re Paulson*, 30 F.3d 1475, 1478-79, 31 USPQ2d 1671, 1673 (Fed. Cir. 1994) (citing *In re Spada*, 911 F.2d 705, 708, 15 USPQ2d 1655, 1657 (Fed. Cir. 1990)). "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987); MPEP §2131.

As an initial matter, claim 1 has been amended. As a result, the presently claimed invention recites a biochip for detecting a small molecule compound comprising a solid support and a conjugate of a carrier and a small molecule compound, wherein the conjugate is immobilized on a surface of the solid support, the carrier is a protein selected from the group consisting of human serum albumin (HSA), bovine serum albumin (BSA), and keyhole limpet hemocyanin (KLH), and the solid support is selected from the group consisting of ceramic, glass, silica, quartz, nylon, plastic, polystyrene and metal.

Applicants respectfully submit that Knecht et al. do not disclose a biochip for detecting small molecules, said biochip comprising a solid support and a conjugate of a carrier and a small molecule on a chip, wherein the carrier is a protein selected from the group consisting of HSA, BSA and KLH. Instead, Knecht et al. only teaches the use of ovalbumin (OVA) and glucose oxidase (GOx) as carrier proteins. This distinction between the presently claimed invention and Knecht et

al. is significant because the characteristics of a carrier protein, such as its molecular weight, charge, functional groups, three-dimensional structure, etc., determine how the carrier protein interacts with the small molecule compound and how effectively they may be conjugated. (*See* Spec. at page 9.) OVA and GOx as disclosed in Knecht et al. have vastly different characteristics from the carrier proteins disclosed in the present application, HAS, BSA and KLH. As such, they are expected to interact differently with the small molecule compounds, and may not be effectively conjugated with the large number of small molecule compounds disclosed in the present invention. (Spec. at pages 8-9.)

Therefore, Knecht et al. do not disclose each and every limitation of the presently claimed invention and thus fail the strict identity test for anticipation. Accordingly, Applicants respectfully request that this rejection be withdrawn.

Claim Rejections - 35 U.S.C. § 103

Knecht et al. in View of Ellis et al.

Claims 1-9 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Knecht et al. as evidence by O'Neil et al. regarding the molecular weights of the compounds utilized by Knecht et al. and in view of Ellis et al. (OA at page 8.) The Examiner acknowledged that Knecht et al. do not teach a substrate comprising an immobilization control immobilized on the surface. (OA at page 10.) In order to address this deficiency, the Examiner cited Ellis et al., which allegedly teach an immobilization control. (*Id.*) The Examiner concluded that it would have been obvious to one skilled in the art at the time of the invention to combine the teachings by Ellis et al. with that by Knecht et al. to arrive at the presently claimed invention. (*Id.*) Applicants respectfully traverse this rejection.

To make a *prima facie* case of obviousness, the teachings of the prior art should have suggested the claimed subject matter to the person of ordinary skill in the art, and all the claim limitations must be taught or suggested in the references cited by the Examiner. *In re Kotzab*, 217

F.3d 1365, 1370 (Fed. Cir. 2000). As articulated by the Supreme Court in a recent case, a combination is obvious if it is no more than the predictable use of known elements according to their established functions; and there was a reason to combine the known elements. *KSR Intl Co. v. Teleflex, Inc.*, 550 U.S. 398 (2007). To make a prima facie case of obviousness, "it remains necessary to identify the reason why a person of ordinary skill in the art would have combined the prior art elements in the manner claimed." *Id.* The initial burden to make a prima facie case of obviousness is on the Examiner. *In re Bell*, 991 F.2d 781, 783 (Fed. Cir. 1993).

As discussed above, Knecht et al. do not disclose a biochip for detecting small molecules, said biochip comprising a solid support and a conjugate of a carrier and a small molecule on a chip, wherein the carrier is a protein selected from the group consisting of HSA, BSA and KLH. Nor do Knecht et al. provide any suggestions for the method claimed in the present invention to use HSA, BSA and KLH as carrier proteins for small molecules. Ellis et al. do not cure such deficiency by Knecht et al. because Ellis et al. do not teach or suggest using HSA, BSA and KLH as carrier proteins for small molecules, either.

Thus, combining the teachings by Knecht et al. with those by Ellis et al. does not teach or suggest all the limitations of the presently claimed invention. Accordingly, the Examiner failed to establish a *prima facie* case of obviousness and this rejection should be withdrawn.

Knecht et al. in View of Li et al.

Claims 1-9 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Knecht et al. as evidence by O'Neil et al. regarding the molecular weights of the compounds utilized by Knecht et al. and in view of Li et al. (OA at page 11.) The Examiner acknowledged that Knecht et al. do not teach a substrate comprising an immobilization control immobilized on the surface. (OA at page 13.) In order to address this deficiency, the Examiner cited Li et al., which allegedly teach an immobilization control. (*Id.*) The Examiner concluded that it would have been obvious to one skilled in the art at the time of the invention to combine the teachings by Li et al.

with that by Knecht et al. to arrive at the presently claimed invention. (*Id.*) Applicants respectfully traverse this rejection.

The standard for a *prima facie* case of obviousness has been discussed. As discussed above, Knecht et al. do not disclose a biochip for detecting small molecules, said biochip comprising a solid support and a conjugate of a carrier and a small molecule on a chip, wherein the carrier is a protein selected from the group consisting of HSA, BSA and KLH. Nor do Knecht et al. provide any suggestions for the method claimed in the present invention to use HSA, BSA and KLH as carrier proteins for small molecules. Li et al. do not cure such deficiency by Knecht et al. because Li et al. do not teach or suggest using HSA, BSA and KLH as carrier proteins for small molecules, either.

Thus, combining the teachings by Knecht et al. with those by Li et al. does not teach or suggest all the limitations of the presently claimed invention. Accordingly, the Examiner failed to establish a *prima facie* case of obviousness and this rejection should be withdrawn.

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue. If it is determined that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the event the U.S. Patent and Trademark office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection

with the filing of this document to Deposit Account No. 03-1952 referencing docket no. 514572002800. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Dated: November 11, 2009

Respectfully submitted,

Electronic signature: /Kun Wang/
Kun Wang
Registration No.: 57,747
MORRISON & FOERSTER LLP
12531 High Bluff Drive, Suite 100
San Diego, California 92130-2040
(858) 314-5435

Exhibit A

GLTaC.com

TRANSLATION CERTIFICATION

Date 11-11-2009

I, (Liyi Chang), being duly sworn, depose and say that:

I am thoroughly conversant with both the Chinese and English languages, and that I have read the attached translation and compared the same with the original document in the Chinese language.

That said translation is a true and complete English version of such original, to the best of my knowledge and belief.

I declare, under penalty of perjury under the laws of the United States, that the foregoing is true and correct.

The documents translated include:

CN200410029590.7

Translator: Liyi Chang

Translator signature: Liyi Chang

Dated: November 11th, 2009

Global Language Translations and Consulting, Inc., 720 W Wackerly St., Suite 8, Midland, MI 48640-2769
Phone: 989.839.5804 - Fax: 989.839.5838 - Email: info@gltac.com

Certificate

The annex to this certificate is a copy of the following patent application submitted to the State Intellectual Property Office

Filing Date: Mar 26th, 2004

Filing number: 200410029590.7

Application type: Invention

The invented product: a method for detecting small molecular compounds and its special bio-chip

Applicant: Beijing Bo'ao Bio-chip Co., Ltd., Tsinghua University

Inventor(s) or designer(s): Sun Yiming, Xing Wanli, Wang Guoqing, Du Hongwu, Zhang Rong, Lu Yuan, Wang Yan and Cheng Jing

Commissioner of the State Intellectual Property Office of People's Republic of China

Wang Jing-chuan

April 12th, 2005

sd-496748

Claims

1. A bio-chip for detecting the small molecular compounds, comprising the solid phase base and the couplet between the small molecular compounds and carrier protein that is attached to the base.

2. The bio-chip for detecting the small molecular compounds according to Claim 1, wherein the said carrier protein is human albumin, bovine albumin, keyhole limpet hemocyanin or ovalbumin.

3. The bio-chip for detecting the small molecular compounds according to Claim 1, wherein the molecular weight of the said small molecular compounds is 1-10000D.

4. The bio-chip for detecting the small molecular compounds according to Claim 1, wherein blank control, negative control, sample preparation reference, chip fixed chemical reference and data normalization reference are attached to the said bio-chip.

5. The bio-chip for detecting the small molecular compounds according to Claim 1, wherein the said solid-phase base is chosen from any of the following: chemically modified ceramic, glass, quartz, porous silicon, plastic, polystyrene, nitrocellulose membrane and metal.

6. The bio-chip for detecting the small molecular compounds according to any of Claims 1 to 5, wherein the said bio-chip is prepared according to the following steps:

1). To couple the small molecular compounds to the carrier protein;

2). To distribute the couplet between the small molecular compounds and the carrier protein onto the chemically modified solid phase base using the automatic spotting device;

3). To obtain the bio-chip through drying.

7. A method for detecting the small molecular compounds, comprising the following steps:

1). To seal the non-spotting area on the bio-chip with confining liquid. The said bio-chip includes the solid phase base and the couplet between the small molecular compounds and carrier protein attached to the base;

2). To add the sample to be tested, its preparation liquid and the preparation liquid of the specific ligand of the small molecular compounds to the spotting area on the bio-chip for reaction;

3). To identify the presence of the small molecular compounds and their contents by detecting the specific ligand of the small molecular compounds.

8. The method for detecting the small molecular compounds according to Claim 7, wherein the specific ligand of the small molecular compounds described in Step 2) is the anti-body of the small molecular compounds or high molecular polymer that may be bonded to the specific ligand of the small molecular compounds.

9. The method for detecting the small molecular compounds according to Claim 7, wherein the specific ligand of the small molecular compounds detected in Step 3) is detected by bonding with the couplet on the specific ligand of the small molecular compounds.

10. The method for detecting the small molecular compounds according to Claim 7, wherein the specific ligand of the small molecular compounds detected in Step 3) is detected by bonding with the couplet on the antibody of the specific ligand of the small molecular compounds.

11. The method for detecting the small molecular compounds according to Claims 9 and 10, wherein the said couplet is fluorescence molecular, enzyme or biotin.

A method for detecting the small molecular compounds and its special bio-chip

Technical Field

The invention deals with a compound detection method and its special equipment and it in particular deals with a method for detecting the small molecular compounds and its special bio-chip.

Prior Art

The bio-chip technology is one of the most influential scientific and technological advancements of great significance since mid 1990s, which falls into the category of new inter-disciplinary technologies that integrate micro-electronics, biology, physics, and chemistry into computer science. A bio-chip means where plenty of biomacromolecules such as nucleic acid fragments, polypeptide molecules and even tissue sections, cells and other bio-samples are solidified onto the surface of the supports (for instance, glass slides, silicon wafers, polyacrylamide gel, nylon membrane and other carriers) in order by means of light directed in-situ synthesis or micro spotting, etc. to form a dense molecular arrangement and react with the target molecules in the bio-sample to be tested and then the strength of the signals after the reaction is detected and analysis in a rapid, simultaneous and highly effectively manner through particular apparatus such as laser scanning confocal microscope or charge coupled camera so as to judge the number of the target molecules in the target molecule. Depending on the probe attached to the chips, the bio-chips may be divided into gene chips, protein chips, cell chips and tissue chips, etc. The chip lab that has witnessed rapid development in recent years is an important branch of the bio-chip science.

Presently, there are primarily two methods available for detecting the small molecular compounds, namely the physico-chemical analysis method and the immune analysis method. The physico-chemical analysis method mainly includes the spectral method, chromatographic method and its combined

technique, and the chromatographic method is the most commonly used one, for instance, high performance liquid chromatography (HPLC), gas chromatography (GC), thin layer chromatography (TLC), etc. The immune analysis includes radioimmunoassay (RIA) method, enzyme-linked immunosorbent assay (ELISA) method and fluoroimmunoassay (FIA) method and the ELISA method is the most commonly used one.

The chromatographic separation system generally consists of separation components, stationery phase and mobile phase. The principle is that according to the differences in the distribution coefficients between the two phases of different components, when the two phases move in relative to each other, the components are distributed repeatedly in the two phases and the purpose of separation and detection is achieved with the flow of the mobile phase. In spite of such advantages as high separation efficiency, good selectivity and strong quantitative and qualitative ability, etc., the chromatographic separation method has obvious shortcomings, such as complex sample preparation process, expensive instruments as well as long time consumed.

Radioimmunoassay of the small molecular compounds is a technique that integrates the immunology, analytical chemistry and synthetic chemistry. ELISA is typical of radioimmunoassay and the ELISA detection of the small molecular compounds is done mainly in the following two manners: one is to coat the antibody of the small molecular compounds to finish the sample detection through the enzyme labeled small molecules; the other is to coat the carrier coupled small molecular antigen to finish the sample detection through the enzyme labeled antigen. In spite of such advantages as rapid analysis and high sensitivity and low cost, the technique has shortcomings like low analysis efficiency, small quantity of information and the detection of a single indicator only.

Description of the Invention

The purpose of this invention is to provide a method for detecting the small

molecular compounds and its special bio-chip.

The bio-chip provided by this invention for detecting the small molecular compounds includes the solid phase base and the couplet between the small molecular compounds and carrier protein attached to the base

Common carrier proteins include HAS, BSA, KLH or OVA; the solid phase base may be any of the following: ceramic, glass, quartz, porous silicon, nylon membrane, plastic, polystyrene, nitrocellulose membrane and metal.

In order to make the detection more convenient and reliable, when designing and preparing the above bio-chip, certain objects of reference may be fixed, including blank control, negative control, sample preparation reference, chip fixed chemical reference and data normalization reference.

The bio-chip provided by this invention for detecting the small molecular compounds is prepared in accordance with the following procedures:

1). To couple the small molecular compounds to the carrier protein;

2). To distribute the couplet between the small molecular compounds and the carrier protein onto the chemically modified solid phase base using the automatic spotting device.

3). To obtain the bio-chip through drying

During the above process, the couplet between the small molecular compounds and the carrier protein is prepared by the conventional method.

The method provided by this invention for detecting the small molecular compounds needs to use the above bio-chip and the process is as follows:

1). To seal the non-spotting area on the bio-chip with confining liquid. The said bio-chip includes the solid phase base and the couplet between the small molecular compounds and carrier protein attached to the base;

2). To add the sample to be tested, its preparation liquid and the preparation liquid of the specific ligand of the small molecular compounds to

the spotting area on the bio-chip for reaction;

3). To identify the presence of the small molecular compounds and their contents by detecting the specific ligand of the small molecular compounds;

The specific ligand of the small molecular compounds used in above Step 2) is generally the antibody of the small molecular compounds or high molecular polymers that may bond to the specific ligand of the small molecular compounds; the specific ligand of the small molecular compounds detected in Step 3) is detected by the couplet on the specific ligand of the small molecular compound or the couplet on the antibody that bonds with the specific ligand of the small molecular compounds; in actual applications, the said couplet is generally fluorescence molecular, enzyme or biotin, etc.

This invention is an organic combination of the bio-chip technology and the RIA analysis method and mainly characterized in: (1) multiple samples: as the micro spotting technology is adopted, one chip may detect multiple samples simultaneously at one time; (2) multiple items: multiple items in the sample tested may be analyzed one by one through one reaction; (3) reliable detection result: step-by-step control is exerted over the entire detection process through the design of the objects of reference, therefore ensuring the reliability of the detection result effectively (4) little consumption of the sample: only about 10μL sample or its preparation liquid is needed. In all, this invention inherits the advantages of the bio-chip like high throughput, large quantity of information and the advantages of the RIA analysis method like easy operation, rapid analysis, high sensitivity and low detection cost, and overcomes the shortcomings of the chromatographic method like expensive instruments, long time consumed as well as the shortcoming of the ELISA detection method like low analysis efficiency. With this invention, the user may finish the qualitative, semi-quantitative or quantitative detection of the small molecular compounds in the sample according to the actual needs. This invention is generally suitable for detecting 1-10000D small molecular compounds.

Description of the Figures

Figure 1 is the detection photo of a negative sample, in which the residual amount of 3 veterinary drugs: enrofloxacin, sulfonamide and streptomycin is lower than the maximum residual amount specified by the State.

Figure 2 is the detection photo of a positive sample of enrofloxacin, in which the residual amount of enrofloxacin is higher than the maximum residual amount specified by the State while the residual amount of sulfonamide and streptomycin is lower than the maximum residual amount specified by the State.

Figure 3 is the detection photo of a positive sample of sulfonamide, in which the residual amount of sulfonamide is higher than the maximum residual amount specified by the State while the residual amount of enrofloxacin and streptomycin is lower than the maximum residual amount specified by the State.

Figure 4 is the detection photo of a positive sample of streptomycin, in which the residual amount of streptomycin is higher than the maximum residual amount specified by the State while the residual amount of enrofloxacin and sulfonamide is lower than the maximum residual amount specified by the State.

The veterinary drug that is tested positive is indicated by white box and the remaining sample points are reference points designed for other veterinary drug samples and to ensure the reliability of the detection result (s).

Preferred Embodiments of the Invention

Example 1. Preparation of the bio-chip for detecting residual veterinary drugs
The preparation process of the bio-chip is stated as follows:

1. To prepare the couplet between enrofloxacin and BSA, couplet between

sulfonamide and OVA, couplet between streptomycin and OVA and negative control, sample preparation reference, chip fixed chemical reference and data normalization reference into spotting liquid at 1.0mg/ml protein concentration using spotting buffer (40% glycol and 60% PBS) and transfer it to the 384-hole plate for spotting.

The method for coupling the small molecular of the above 3 veterinary drugs and the carrier protein is stated as follows:

Synthesis method of the enrofloxacin couplet: 1) weigh 1200ml enrofloxacin hydrochloride accurately and dissolve it in 1.0ml pure water, add 2mol/l sodium hydroxide solution to adjust the ph value to 6.0 and place it into a refrigerator at 4℃ for cooling for 30 minutes. Then, add DCC and NHS solutions for reacting for 0.5h. 2). Weigh 1.0g BSA and dissolve it in 0.2mol/l phosphate buffer, and then add the reaction liquid in Step 1 for placing overnight at 4℃. 3) Put the prepared full antigen solution into the dialysis bag, perform dialysis with phosphate buffer for 5 days (the number of times of liquid replacement is no less than 12), and then store in separate packages at —20℃.

Synthesis method of the sulfonamide couplet: 1) weigh 500mg sulfonamide liquid accurately and dissolve it in 50μL DMF, add 50% glutaraldehyde for reaction and place it into a refrigerator at 4℃ for reacting for 50 minutes. Then, add sodium carbonate solution for reacting for 1h and measure the ph value to be 8—9 . 2). Weigh 1.0g OVA and dissolve it in 0.2mol/l phosphate buffer, and then add the reaction liquid in Step 1 for placing overnight at 4℃. 3) Put the prepared full antigen solution into the dialysis bag, perform dialysis with phosphate buffer for 5 days (the number of times of liquid replacement is no less than 12), and then store in separate packages at —20℃.

Synthesis method of the streptomycin couplet: 1) weigh 500mg streptomycin sulfate accurately and dissolve it in 500μL pure water, add 2.0g methoxylamine for reaction at room temperature for 3h after dissolution. Then,

add sodium carbonate solution for reacting for 1h and measure the ph value to be 7.5 before adding 600mg DCC for reacting for 2 hours. 2). Weigh 1.0g OVA and dissolve it in 0.2mol/l phosphate buffer, and then add the reaction liquid in Step 1 for placing overnight at 4℃. 3) Put the prepared full antigen solution into the dialysis bag, perform dialysis with phosphate buffer for 5 days (the number of times of liquid replacement is no less than 12), and then store in separate packages at —20℃.

2. To distribute the prepared spotting liquid onto the glass slide in accordance with certain dot matrix arrangement method through the automatic spotting device. Each chip consists of 10 (5 columns X 2 rows) arrays, each of which consists of 36 (6 columns X 6 rows) sample points, which are 400μm apart from each other. Each array forms an independent reaction chamber;

3. To perform vacuum drying of the spotted chip;

4. To package the chip in vacuum for storage at 4℃.

The bio-chip prepared according to the above method may also perform qualitative, semi-quantitative or quantitative analysis of enrofloxacin, sulfonamide and streptomycin in the samples simultaneously.

Example 2: Detection of Residual Veterinary Drugs through the Bio-chip

1. Chip blocking: block the aforesaid prepared bio-chip with 10% normal goat serum solution for blocking at 37℃ for 30 minutes;

2. Clean and dry the chip: take out the chip and place it into a box, clean by shaking with PBST solution (containing 0.5％Tween—20) shaker for 5 minutes, put the chip into the centrifuge for centrifuging at 1000rpm for 1 minute before it is centrifugally dried;

3. First antibody reaction: take the sample to be tested (or its preparation) and the antibody mixture (the concentration of each antibody is 1μg/ml) of enrofloxacin, sulfonamide, and streptomycin of equal volume, add them to the

micro centrifuge tube, mix them evenly, suck 20μL and add it to the reaction chamber of the chip for reaction at 37℃ for 30 minutes;

4. Second antibody reaction: clean and dry the chip using the method described in Step 2 and then add 20μL fluorescence labeled goat anti-mouse IgG (the concentration is 1μg/ml) for reaction at 37℃ for 30 minutes;

5. Chip scanning and data processing: clean and dry the chip using the method described in Step 2 and then perform chip scanning and data processing. The result is given in 1-4.5.

Description of the result: since the principle of this method is competitive immunoassay, the weaker the signal of the sample points of the chip, the higher the residual amount of the veterinary drug in the sample.

The technical indicators that may be achieved through the chip detection system of the small molecular compounds described in this invention are compared with the maximum residual limits (MRL) specified by the State in the table below:

	Sensitivity(ng/g)	Linear range (ng/g)	MRL(ng/g)
Enrofloxacin	1	1～50	100
sulfonamide	0.5	0.5～20	25
streptomycin	5	5-200	200

Note: the values of MRLs in the table above are the minimum values of the allowable MRLs of various types of samples.

As the sensitivity of this system is far greater than the MRL specified by the State Competent Authority, the sample preparation liquid shall be diluted before detecting the actual samples.

The contents of the small molecules of the veterinary drugs in Figures 1-4 are shown as follows:

	Enrofloxacin (ng/g)	Sulfonamide (ng/g)	Streptomycin (ng/g)
Figure 1	0	0	0
Figure 2	200	0	0
Figure 3	0	50	0
Figure 4	0	0	400

Figures Attached to the Specifications

Figure 1

Figure 2

Figure 3

Figure 4